ENDOCRINE RESISTANCE IN ADVANCED BREAST CANCER

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Although about half of patients with advanced breast cancer show either an objective response or disease stabilization on first line endocrine therapy, virtually all eventually relapse. Few of those patients that fail to respond to first-line treatment, respond to challenge with a second agent. In most cases, the cause of this de novo resistance appears to be the presence of only very low levels of oestrogen receptor (ER) and presumed growth dependence on other pathways. Patients who develop acquired resistance after an initial response, have approximately a 50% chance of responding to a further agent. The most frequently used first-line agent is tamoxifen, and the understanding of acquired endocrine resistance mainly relates to this agent. Selection of ER-ve clones of cells does not appear to occur frequently, and there is little clinical evidence to support the role of ER variants or mutants. There is evidence, however, that in some patients the intratumoural concentration of tamoxifen is substantially reduced at relapse, despite no change in plasma levels.

Resistance to endocrine therapy in patients with advanced breast cancer can be divided into two groups: (i) those patients who show a complete resistance to all endocrine treatments and can be considered as de novo or intrinsically resistant (ii) those patients who respond to endocrine therapy initially, but relapse with acquired resistance. Unfortunately, in the advanced setting, all responding patients eventually relapse (unless they die of intercurrent disease). Such relapse with acquired resistant disease as well as being acutely problematic also provides opportunities for further treatment since generally such patients show a good likelihood of responding to further endocrine manipulations. Thus this is a complicated scenario, with non-cross resistance to different agents occurring in many patients, but some relatively clear lessons may be learnt.

Over recent years increased efforts have been made to try to understand the molecular mechanisms whereby resistance develops. This is important for the multitude of drugs which are increasingly available to be applied optimally, and for a rational approach to new drug development to be taken. The most fruitful studies have been those which have pursued the study of biochemical changes within tumour biopsies while recording the clinical pattern of response and resistance. These studies are relatively few in number and the most powerful have involved the study of resistance to endocrine therapy of the primary tumour. The mechanisms elucidated are likely, however, to apply to metastatic disease.

It is helpful when trying to understand resistance mechanisms better, to distinguish between tamoxifen resistance and resistance to oestrogen withdrawal. The bulk of the data relate to tamoxifen resistance largely because of the great popularity of this agent but its complicated pharmacology makes it particularly difficult to establish probable mechanisms.

Tamoxifen

It is well known that approximately one-third of unselected patients will show an objective response to tamoxifen when no prior treatment has been given. It is notable, however, that there is a substantial further proportion (15–25%) who while not showing a response according to the objective criteria of the UICC, do destabilise on treatment. It is clear that this group of patients derive some

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Table 1

Negative correlation of ER and EGFr in primary breast carcinomas. The data are subdivided according to their response to primary tamoxifen therapy where given.

	Responders		Non-responders		Adjuvant		Total	
EGFr	+	−	+	−	+	−	+	−
ER+	1	15	0	3	0	16	1	34
ER−	0	2	7	8	8	11	15	21

benefit from this stabilisation since the prognosis of patients that stabilise on endocrine therapy is similar to those with an objective response (1). Thus, probably about 50% of patients "respond" to tamoxifen and a similar proportion show de novo resistance.

In general, de novo resistance is associated with low levels of oestrogen receptor (ER), conventionally described as ER negativity, such that the original tumour appears already to be independent of oestrogen in growth. This ER negativity is frequently associated with increased growth factor receptor expression which may provide an alternative drive to the tumour's growth (e.g. EGFr, Table 1). When responders to tamoxifen relapse, about half of them respond to a further endocrine manipulation. Although a few of the other half may respond to challenge with a third-line agent, the majority are totally resistant to further endocrine challenge. In trying to understand mechanisms which might explain this bifurcation, there are certain molecular changes which would be expected to lead to a tamoxifen-specific resistance and others which will be expected to lead to pan-endocrine resistance. These are discussed separately below.

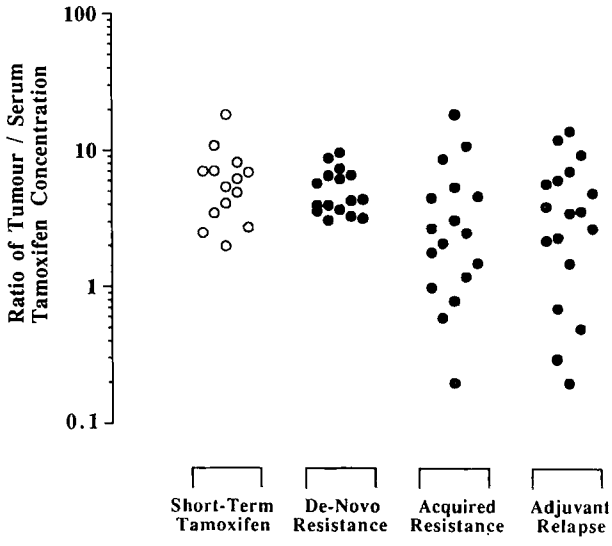

Fig. 1. Ratio of intratumoural tamoxifen (ng/g) to serum tamoxifen (ng/ml) in 51 patients with clinically defined tamoxifen resistance and in 14 patients with primary breast cancer treated with short-term tamoxifen for at least 2 weeks prior to surgery (2).

Tamoxifen-specific resistance

(a) Intratumoural tamoxifen concentrations: We have established that a proportion of primary breast cancer patients who showed acquired resistance to tamoxifen but not those which showed de novo resistance had decreased concentrations of tamoxifen in their tumour at relapse (2). In some 4 patients with acquired resistance or relapse upon adjuvant therapy the intratumoural tamoxifen levels were more than ten-fold lower than in patients shortly after starting treatment (Fig. 1). Such a major difference could be expected to be of reduced efficacy. At present, it is not known why these low tamoxifen concentrations occur. Essentially, three explanations could be proposed: (a) reduced uptake by the tumour, (b) increased intratumoural metabolism (c) increased extrusion of tamoxifen.

Reduced uptake might be due to increased binding of tamoxifen in the extracellular compartment. We have investigated this by measuring the binding of tamoxifen to plasma proteins in the serum and compared this with tamoxifen concentrations in tumours of the same patient (3). We have found no support for increased protein binding being a significant mechanism for reducing intratumoural concentrations.

Metabolism of tamoxifen by tumours is only a theoretical possibility. Metabolites certainly exist in the tumour but in our (unpublished) studies of incubates of tamoxifen with MCF7 cells, no significant metabolism was seen.

The concept of increased extrusion is attractive since this would be parallel to the multidrug resistance syndrome in which the membrane pump P-glycoprotein apparently has a mechanistic role. A significantly greater expression of P-glycoprotein has been found in tamoxifen-resistant tumours compared with tumours which responded to primary tamoxifen (4). However, it has been demonstrated that transfection of P-glycoprotein into MCF-7 cells does not render them tamoxifen resistant (5). Thus, although a pump may be involved, the balance of evidence is against this being P-glycoprotein.

(b) Increased agonist activity: There are data from a number of clinical studies, as well as a number of model systems which indicate that tamoxifen can act as an agonist at relapse. For example, there does seem no doubt that some patients show clinical responses on withdrawal of

tamoxifen (6, 7). Further clinical data supporting this are derived from studies of tamoxifen in premenopausal women in whom at relapse GnRH agonists were introduced either with or without withdrawal of tamoxifen (8, 9). Although this was not a randomized comparison, the good response rate (6/8) in those who had tamoxifen withdrawn is in striking contrast with the total lack of responses (0/14) in those patients in whom tamoxifen treatment was maintained. The significance of this agonist activity is further suggested in studies in vitro in which breast cancer cells from several effusions could be growth-supported by tamoxifen but not pure antioestrogen (10). Also, Jordan's group have recorded the growth support of MCF-7 human breast cancer xenografts by tamoxifen after initial tumour relapse (11). The molecular explanation for this increased agonism is not clear, although suggestions that certain co-activators of the oestrogen response mechanism, such as the recently characterized protein SRC1 (steroid receptor co-activator 1), could influence the balance of agonist/antagonist activity (12), indicate that this particular group of compounds merits substantial investigation.

It should be noted that the occurrence of withdrawal responses to tamoxifen can be a complicating factor when interpreting the response rates to endocrine agents used second-line on tamoxifen relapse.

Pan-endocrine resistance

(a) Growth factor pathways: There is substantial evidence to indicate the negative correlation with ER levels of c-erbB2 and particularly EGF receptor (Table 1), and the expression of these growth factor receptors is associated with poor response to endocrine treatment (13, 14). However, in our investigations, there is no evidence whatsoever for increased expression of these growth factor receptors in those patients who show acquired resistance. Of 15 patients who responded initially to tamoxifen treatment, all were c-erbB2 negative at presentation and remained so at relapse on tamoxifen, and while one was EGFr positive at presentation, all were EGFr negative at relapse. One cannot, however, exclude the possibility that down-stream events in signal transduction from these growth factor receptors may be perturbed in tamoxifen resistance and lead to that resistant phenotype.

(b) Oestrogen receptor loss, mutations and variants: For many years it was considered that the selection pressure of tamoxifen might lead to the outgrowth of an ER-ve tumour. The data in the main refute this. A small percentage of patients may be rendered ER-ve at relapse and the absolute levels of ER are somewhat lower (15) (Table 2). However, the bulk of tumours remain ER + ve with levels which are generally considered consistent with good response to endocrine manipulation.

Oestrogen receptor mutations have been generated in the laboratory which lead to loss of signal transduction activity or in some cases enhanced agonist response to tamoxifen. However, we have not found these receptor mutants in 20 tamoxifen-resistant (or 20 non-resistant) breast carcinomas (unpublished) and we, therefore, feel that oestrogen receptor mutation can be discounted as a major mechanism for resistance.

Several groups, including our own, have reported the existence of ER variants in which the mRNA differs from a non-mutated oestrogen receptor gene. Particular interest has been directed at a variant which lacks the exon 5 and in some systems is seen to be constitutively active despite lacking the ligand-binding domain and therefore not being able to bind either oestrogen or tamoxifen (16, 17). In a large population of tamoxifen-resistant tumours, we found no increase in the levels of this variant ER mRNA in tamoxifen resistant tumours (18). These data indicate this variant to be of little or no importance other than in a very small percentage of tamoxifen-resistant patients.

Oestrogen deprivation

Oestrogen deprivation is achieved differently in pre- and postmenopausal women. The bulk of investigations of resistance have been directed to the understanding of aromatase inhibitors which are used exclusively in women lacking ovarian function. Little is known about resistance

Table 2

Change in ER status between paired biopsy samples taken before tamoxifen treatment and at relapse. Measurements were made by immunocytochemistry. Positivity was defined by H-score ⩾20. Patient groups are divided according to their response to primary tamoxifen treatment where given (15)

	Responders		Non-responders		Adjuvant	
	Ore	Relapse	Pre	Relapse	Pre	Relapse
Number ER+/ER−	16/2	11/7	3/17	0/20	18/16	10/24
H-score	70	66	99	0	103	57
Wilcoxon-matched pairs p-value	0.12		0.008		0.0002	

to GnRH agonists, although we found that response to a GnRH agonist was followed by further response, in 4 of 6 patients, on addition of an aromatase inhibitor (19). This suggests that in these secondary responders, the treated tumour was resistant to the prevailing low-oestrogen environment, but sensitive to further oestrogen deprivation.

An analogous situation exists with aromatase inhibitors in postmenopausal patients: we have noted that the use of 4-hydroxyandrostenedione followed by aminoglutethimide (AG) as additional therapy suppresses oestrogen levels further, and is also associated with further clinical responses (20). Recently, Santen's group (21) have indicated that oestrogen deprivation to the MCF7 human breast cancer cell line both in vitro and in vivo, leads to a substantially enhanced sensitivity to oestrogens which can then stimulate proliferation at levels as low as 10^{-15}M. While this importantly provides parallels in the in vitro setting and supports the possibility of increased oestrogenic sensitivity as a mechanism of resistance, the molecular explanation for this is unknown. One possibility could be the increased expression of an oestrogen receptor variant, which has being observed to be present in human tumour specimens and has a sensitivity to oestradiol of about 100-fold greater than wild-type receptor (22).

There has been a tendency over recent years to develop increasingly potent aromatase inhibitors with the most recent triazole compounds effectively, obliterating aromatase activity (23) and yielding plasma oestrogen concentrations below the sensitivity of conventional immunoassays (2–3 pmols/L). However, it remains to be demonstrated whether the application of these agents (or compounds such as pure antioestrogens) may provide an improved disease control in the advanced setting. The examples above demonstrate that stepwise oestrogen deprivation can continue to control the disease and it is possible that the application of agents which yield essentially a complete oestrogen deprivation as first-line therapy may advance the development of a pan-endocrine resistant phenotype. Clinical trials are urgently required to address this question in a systematic manner.

Further explanations for the development of resistance to aromatase inhibition have been suggested. For example, Miller and O'Neill (24) indicated that only those patients who have aromatase in their primary tumour respond to AG and these data have been supported by the work of Bezwoda and colleagues (25). The measurement of aromatase activity is, however, difficult and cumbersome and confirmation of this result on a widespread basis remains to be achieved. Acquired resistance could also possibly occur with the increased expression of aromatase by selection of cells which have high levels of aromatase and therefore might have a growth advantage under the selective pressure of an aromatase inhibitor. There are limited data which support this for AG (24), but it requires further study with the new endocrine agents. There are also some data to indicate that different inhibitors react differently to mutant forms of aromatase in the experimental laboratory setting (26). There are, however, no data to support the existence of these aromatase enzyme mutations in tumours (27) and therefore this can probably be discounted as a differential determinant of sensitivity.

Lastly, the host may itself show an adaptation to treatment with aromatase inhibition by increased plasma oestrogen levels. The only support for this mechanism is provided by our study conducted many years ago in which increased levels of oestrone were observed, just prior to relapse (28). These levels, however, did not approach pretreatment levels and were probably explained by a stress response within the host.

Certain cross-over data in which AG and tamoxifen have been compared suggest that resistance to second-line treatment occurs more frequently when the aromatase inhibitor is used first-line (29). This could be due to sensitivity to oestrogen simulation being maintained by the agonist activity of tamoxifen, while resistance to "pure" oestrogen deprivation might lead to the loss of the mechanism of response to oestrogen and therefore tamoxifen. It might in fact be expected that relapse on an aromatase inhibitor, in contrast to relapse on tamoxifen, could be due to the development of an ER-ve clone of cells, but to date the data do not support this (30).

Conclusion

Some understanding of the molecular mechanisms of resistance is beginning to be achieved. There are a multitude of possible mechanisms with support for some of these in clinically derived specimens. There are, however, many further areas to investigate prior to the achievement of a systematic understanding of this area. Only then will we be near to a rational selection of optimal therapy for individual patients.

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